CLAIMS

1. A method for detection of an analyte a in a fluid sample, characterized in that it comprises the following steps:

1) saturating a solid support comprising, on at least part of its surface, at least one trifunctional reagent (tripod Y) comprising the following three functional poles:

i) a luminescent group (L),

ii) a molecule (B) chosen from the analyte a, an analog of the analyte a or a fragment of the analyte a; and

iii) a function that provides attachment of said trifunctional reagent to the surface of said solid support,

with a receptor for the analyte a, said receptor being labeled with a compound (Q) (receptor-Q) that quenches the luminescence of the group L, so as to form a complex C between said molecule (B) and said receptor-Q;

2) bringing the solid support obtained in step 1) into contact with a fluid sample that may contain the analyte a to be detected;

3) measuring the intensity of the signal emitted by the group L, which is proportional to the amount of analyte a present in the fluid sample; and

4) regenerating the solid support by bringing said solid support into contact with the receptor-Q.

2. The method as claimed in claim 1, characterized in that several types of tripods Y that differ from one another through the nature of the molecule (B) that they comprise are attached to distinct and known zones of the solid support.

3. The method as claimed in claim 1 or 2, characterized in that steps 3) and 4) are carried out continuously.

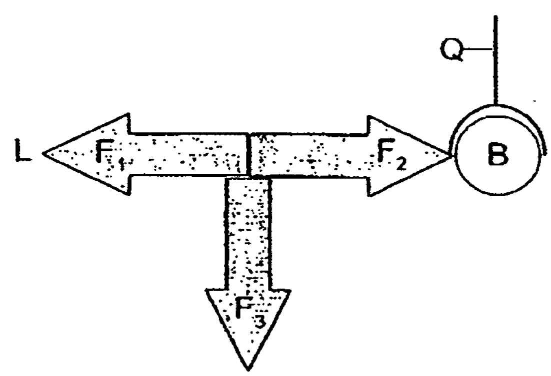
4. The method as claimed in any one of the preceding claims, characterized in that the solid support is chosen from glasses, plastics, ceramics, metals and metalloids.

5. The method as claimed in any one of the preceding claims, characterized in that the support is in the form of a tube, a capillary, a plate or a bead.

6. The method as claimed in any one of the preceding claims, characterized in that the fluid sample consists of water, a liquid biological medium, or a liquid medium containing dissolved gaseous molecules or molecules originating from solid samples.

7. The method as claimed in any one of the preceding claims, characterized in that the intensity of the signal emitted during step 3) is determined by means of a luminescence detector.

8. The method as claimed in any one of the preceding claims, characterized in that the complex C formed at the end of the saturation step 1) is chosen from the complexes of formula (I) below:



(I)

in which:

- the arrows represent the structure of the backbone of the tripod Y, which is a linker arm consisting of a peptide, nucleotide or glucoside chain or of a saturated or unsaturated, linear or branched hydrocarbon-based chain; said chains being optionally substituted, interrupted and/or ended with one or more hetero atoms, such as N, O or S, and/or with one or

more amino acids, and comprising three reactive chemical functions F_1, F_2 and F_3;

- L represents a luminescent group covalently bonded to the tripod Y by means of the reactive chemical function F_1;

- B represents an analyte a, a structural analog of an analyte a or a fragment of an analyte a to which is noncovalently and reversibly attached a receptor specific for the analyte a, said receptor being labeled with a compound Q; the molecule (B) being covalently bonded to the tripod Y by means of the reactive chemical function F_2;

- Q represents a compound that quenches the luminescence of the group L;

- F_3 represents a reactive chemical function that can allow the attachment of the tripod Y to the surface of the solid support.

9. The method as claimed in claim 8, characterized in that the functions F_1, F_2 and F_3, independently of one another, provide:

i) either a direct linkage via a corresponding chemical function present on the luminescent compound, the molecule (B) or the solid phase;

ii) or an indirect linkage, and in this case, the linkage is carried out by coupling, to at least one of the functions F_1, F_2 and/or F_3, a molecule M_1 capable of forming a complex with a molecule M_2 attached beforehand to at least part of the surface of the solid phase, to the molecule (B) and/or to the luminescent group.

10. The method as claimed in claim 8 or 9, characterized in that the functions F_1, F_2 and F_3, which may be identical or different, are chosen from the following functions: thiols; amines; alcohols; acid functions; esters; isothiocyanates; isocyanates; acylazides; sulfonyl chlorides; aldehydes; glyoxals; epoxides; oxiranes; carbonates; imidoesters; carbodiimides; maleimides; nitriles; aziridines; acryloyl; halogenated derivatives; disulfide groups;

phosphorus-containing groups; diazo; carbonyldiimidazole; hydrazides; arylazides; hydrazines; diazirines; magnesium compounds; lithium compounds; cuprates; zinc compounds and unsaturated systems.

11. The method as claimed in claim 10, characterized in that the functions F_1, F_2 and F_3 are chosen from amine functions of formulae $R-NH_2$, R-NH-, $(R)_3-N$, R-NH-OR and NH_2-OR; alcohol functions R-OH; and halogenated groups of formula R-X with X representing a halogen atom; it being understood that, in said formulae, R represents an alkyl, aryl, vinyl or allyl radical.

12. The method as claimed in any one of the preceding claims, characterized in that the luminescent groups are chosen from fluorescein (sodium fluoresceinate) and its derivatives; rhodamine and its derivatives; diaminidophenyl indo (DAPI); acridine; fluorescent dyes with reactive amines; the fluorescent dyes sold under the brand names Bodipy®; the dyes Cascade Blue, Cy2, Cy3, Cy3.5, Cy5, Cy5.5 and Cy7, Dabcyl® and Edans®; eosin; erythrosine; 6-Fam and Texas Red.

13. The method as claimed in any one of the preceding claims, characterized in that the receptors are chosen from antibodies in whole, fragmented or recombinant form, biological receptors, nucleic acids, peptide nucleic acids, lectins, transporter proteins, chelates and synthetic receptors.

14. The method as claimed in any one of the preceding claims, characterized in that said receptor exhibits greater affinity for the analyte a than for the molecule (B).

15. The method as claimed in any one of the preceding claims, characterized in that the quenching compound (Q) is chosen from rhodamine and its derivatives, the fluorescent compounds mentioned in claim 12, nonfluorescent molecules chosen from the compounds sold under the brand names Black Hole

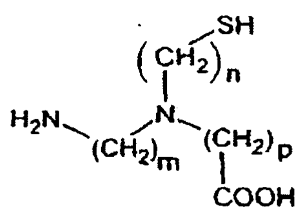
Quencher® 1, 2 and 3, Nanogold Particules®, Eclipse Dark Quencher®, Elle Quencher®, malachite green, and the dyes QSY® 7, QSY® 9 and QSY® 21.

16. The method as claimed in any one of claims 8 to 15, characterized in that the complexes of formula (I) are chosen from the compounds in which:

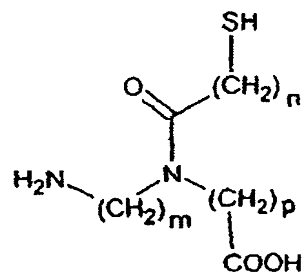
i) (B) is chosen from peptides, proteins, oligonucleotides, sugars and peptide nucleic acids,

ii) L is fluorescein, and

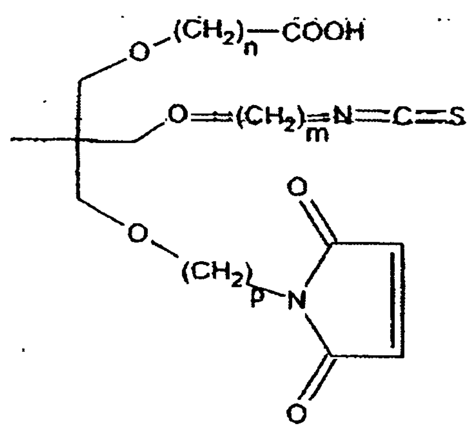
iii) the backbone of the tripod Y is chosen from the structures Y_1 to Y_3 below:



(Y_1)



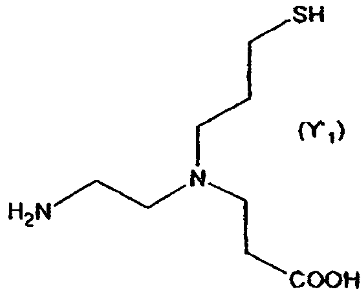
(Y_2)



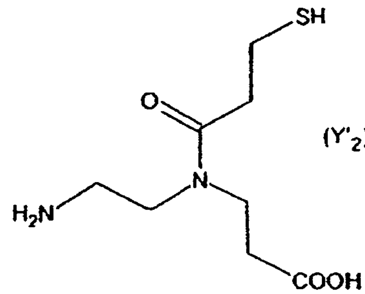
(Y_3)

in which n, m and p, which may be identical or different, are integers between 1 and 20 inclusive.

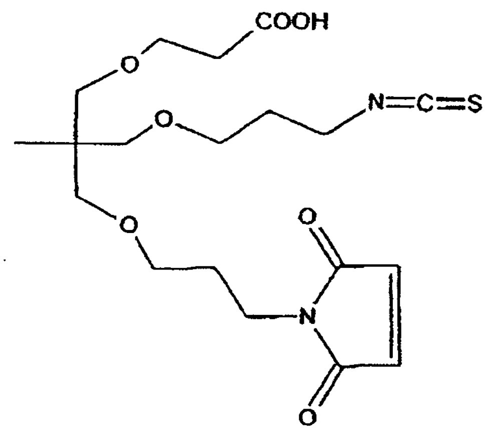
17. The method as claimed in claim 16, characterized in that the structures Y_1 to Y_3 are chosen from the compounds of formulae (Y'_1) to (Y'_3) below:



(Y'_1)

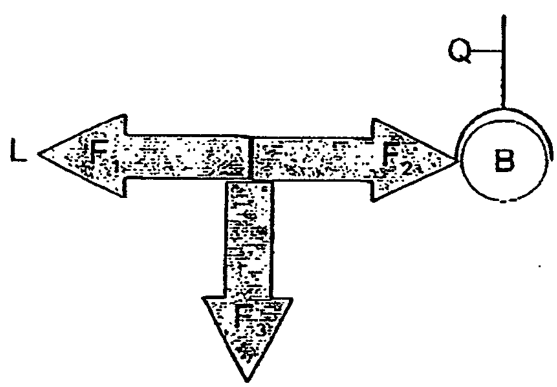


(Y'_2)



(Y'_3)

18. A complex C, characterized in that it corresponds to formula (I) below:



(I)

in which L, B, Q, the arrows and F_1, F_2 and F_3 are as defined in any one of claims 8 to 17.

19. Use of at least one complex C of formula (I) as defined in any one of claims 8 to 16, in a method for continuous heterogeneous-phase detection of an analyte <u>a</u> in a fluid sample.

20. A device for continuous heterogeneous-phase detection of at least one analyte a in a fluid sample, said device being characterized in that a fluid sample to be analyzed is integrated into a medium forming a stream that flows over at least one solid support at the surface of which is attached at least one tripod Y as defined in claim 1 and specific for the analyte a to be detected, a luminescence detector placed opposite the solid support is coupled to a valve control that is controlled by a threshold of intensity of signal emitted by the detector and which triggers, for a given period of time, the opening of a reservoir containing a receptor-Q capable of forming a complex with the tripod Y, this reservoir being linked to the support via a feedback loop which comes in upstream of the solid support to which the tripod Y is attached, in order to saturate and/or regenerate the latter with receptor-Q by passage in the stream and complexation on the tripod Y.

21. The device as claimed in claim 20, in which the luminescence intensity values are monitored and secondarily translated into an amount of analyte a by a calculation system coupled to the luminescence detector.

22. The device as claimed in claim 20 or 21, in which an event marker is placed in the feedback loop in order to signal a variation in intensity of the signal above a predetermined value.

23. The device as claimed in any one of claims 20 to 22, in which the solid support is a capillary coupled to the environment containing the sample to be analyzed, the coupling being carried out either by means of a round-bottomed capture flask in which the sample sparges in a medium corresponding to that of the flow stream, or by means of a flexible pipe.

24. The device as claimed in any one of claims 20 to 23, in which the stream is entrained by means of the low pressure produced by a pump, a piston, or equivalent.

25. The device as claimed in any one of claims 20 to 24, characterized in that it is equipped with a round-bottomed capture flask and with a sparging system for collecting samples in gaseous form and for solubilizing the constituents to be detected that they contain.

26. The use of a device as defined in any one of claims 20 to 25, for detecting the presence of an analyte a in a natural or industrial medium.

27. The use as claimed in claim 26, characterized in that the device is used in lakes, rivers, swimming pools, factories, purification plants, or ventilation or air-conditioning systems.